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DATA EVALUATION RECORD

STUDY TYPE: Delayed Neurotoxicity- Hen

OPPTS NUMBER: OPPTS 870.6100 OPP Guideline Number: §81-7

DP BARCODE: D234794 SUBMISSION CODE: S819418
P.C. CODE: 08102 MRID NO.: 00126254

TEST MATERIAL (PURITY): Pirimiphos-methyl (93.5% a.i.)

CITATION: Roberts, N.L.; Fairley, C; Almond, R. N. and Prentice, D.E. 1983; The sub-chronic delayed neurotoxicity of pirimiphos-methyl to the domestic hen, Imperial Chemical Industries P.L.C., Central Toxicology Laboratory, Cheshire, U.K., Study No. ICI 411NT/821118, February 4, 1983, MRID: 00126254, EPA Acc. No. 071451, Unpublished.

SPONSOR: ICI Americas, Inc., Wilmington, DL.

EXECUTIVE SUMMARY: Groups of 10 hens received 0.5, 1.0, 2.5, 5.0 and 10.0 mg/kg/day pirimiphos-methyl (purity 93.5%) by gavage daily for a total of 90 doses. Two Additional groups received 5.0 or 10.0 mg/kg/day (total of 90 doses) followed by a recovery period of 30 days. Control groups consisted of untreated control and vehicle control. The positive control group received TCOP (7.5 mg/kg/day).

Treatment-related findings were noted at 5.0 and 10.0 mg/kg/day. These consisted of mortality (4/20 and 8/20 at 5.0 and 10.0 mg/kg/day, respectively), decrease in body weight, food consumption (at 10.0 mg/kg/day only), and dose related increase in the severity of clinical signs of toxicity including quietness, weakness, sluggish movements, stumbling, leg stiffness, ruffled feathers, exaggerated leg movements, unsteadiness and wing drooping. Mild and less frequent signs were noted at 1.0 and 2.5 mg/kg/day; none were seen at 0.5 mg/kg/day. At 10 mg/kg/day, the clinical signs were seen even during the recovery period. Histopathology revealed neuropathological changes (Grade III) in one out of 6 hens from non-recovery period group that received continuous dosing; histopathology was not performed on the recovery group. No NTE measurements were made. The birds in the recovery groups regained their body weight. TCOP treated controls had well defined ataxia, decreased body weight and food consumption, and axonal degeneration in the spinal cord. **Based on the limited evidence of histopathological lesions, the LOEL for delayed neurotoxicity was 10 mg/kg/day. The NOEL was 5 mg/kg/day.**

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This study is classified as Unacceptable/Guideline and does not satisfy the requirements for a delayed neurotoxicity study (§81-7) in hens because of intermittent dosing of some animals. At 10 mg/kg/day, the non-recovery group animals had less severe and less frequent signs of toxicity compared to those that were seen in recovery animals. At 10 mg/kg/day (non-recovery group) some animals that died earlier did not receive sufficient dosing and therefore, the histopathological examination did not revealed treatment-related effects.

The subchronic delayed neurotoxicity of pirimiphos-methyl to the domestic hen.

Huntingdon Research Center, # ICI 411NT/821118, Feb. 4, 1983, EPA Acc. No. 071451, Tab C1.

10 groups of 10 hens (Gallus gallus domesticus), aged 14 months (approx.), were grouped as follows:

Group	
1	Vehicle control (corn oil)
2	Untreated control
3	Positive control (TOCP, 7.5 mg/kg/day)
4	Pirimiphos-methyl 0.5 mg/kg/day
5	Pirimiphos-methyl 1.0 mg/kg/day
6	Pirimiphos-methyl 2.5 mg/kg/day
7	Pirimiphos-methyl 5.0 mg/kg/day
8	Pirimiphos-methyl 10.0 mg/kg/day
9*	Pirimiphos-methyl 5.0 mg/kg/day
10*	Pirimiphos-methyl 10.0 mg/kg/day

The pirimiphos-methyl used for this study was from issue 002 or RS/78/G. The purity was not stated. The individual doses were determined on the basis of the daily weight of each bird. Each bird received a total of 90 doses by gavage. In some cases for the higher doses, it was necessary to allow a few days between dosing in order to prevent killing the birds. *Groups 9 and 10 were allowed 90 days to recover from any lesions which might have been induced by the pirimiphos-methyl treatment.

Results:

1. Stability of pirimiphos-methyl in corn oil. The chemical was shown to be stable in corn oil for up to 10 days. The concentrations of pirimiphos-methyl were shown to be within 8% of the nominal concentrations.

2. Mortalities - 18 of the birds died during the 90-day dosing period. None were in the untreated or vehicle control groups. 4 of 10 of the birds dosed with 10 mg/kg (in each group) died.

A single bird dosed with 1.0 mg/kg of pirimiphos-methyl died; 3 birds in the 2.5 mg/kg group died or were sacrificed in extremis; 4 of (20) birds dosed with 5 mg/kg died or were

includes recovery group

sacrificed in extremis. During recovery, an additional 5 birds died: 4 in the group dosed with 5.0 mg/kg and 1 in the group dosed with 10 mg/kg.

3. Clinical signs of toxicity. The birds dosed with pirimiphos-methyl showed signs of abnormalities in their gait which included "quietness, weakness, sluggish movements, ruffled feathers, wing drooping, stumbling, unsteadiness, leg stiffness, and exaggerated leg movements." None of these signs were reported in the group dosed with 0.5 mg/kg/day. Other signs reported included gurgling noises.

Ataxia was assessed using the criteria appended. The bird was held by its wings 0.5 to 1.0 m above a passage floor and released. The bird then walked toward its pen and had to jump up to a crate (32 cm high) and off again to reenter its pen.

Using this method of assay, no consistent signs of ataxia were reported by the laboratory results in any of the negative control groups or the hens treated with pirimiphos-methyl. The hens treated with TOCP developed the ataxia as expected.

Inspection of Appendix 6B of the study report indicates that several of the signs listed in the ataxia assessment were evident in the hens treated with pirimiphos-methyl (these are listed and underlined in the first paragraph of this section above).

4. Pathology. The birds were sacrificed by pentobarbitone injection and were perfused with 10% neutral buffered formalin while under anethesias. The head, spinal column and sciatic nerves were preserved in the formalin and the following samples were taken for histology:

Optic nerves
Olfactory nerves
Forebrain
Mid- and hind brain
Upper cervical spinal cord)
Lower cervical spinal cord) one cross section and two longitudinal sections
Thoracic spinal cord) at each level
Lumbar spinal cord)
Dorsal root ganglia
Proximal sciatic nerve
Distal sciatic nerve
Tibial nerve (distal branches)
Myoneural junction (taken at termination and from some sporadic mortalities but not examined)

APPENDIX 5

Key to points scoring system used in ataxia assessment

0. No ataxia.

1. Doubtful; slight inco-ordination, not always apparent.

2. Slight inco-ordination; occasional stumbling or wing-dropping especially after exertion.

3. Frequent inco-ordination or stumbling, especially on alighting or after exertion.

4. Staggering gait, tail and leg reflexes may be affected; bird lands awkwardly.

5. Continuous staggering gait, bird rests often; tail and leg reflexes usually noticeably affected.

6. Bird stands for short periods only, normally moves by shuffling on hocks; tail and leg reflexes usually noticeably affected.

7. As 6; weakening limb movements; reflexes markedly affected.

8. Bird unable to stand, weak limb movements; tail and leg reflexes virtually non-existent.

Zeroxed from study report

Zeroxed 1 m. study report

APPENDIX 11

(continued)

Key to grading scheme in individual bird reports and summary tables

Grade I	No white matter abnormality detected.
Grade II	Disruption or fragmentation of occasional axons. Myelin abnormalities are rare. In general, on any slide of the spinal cord (two longitudinal and one transverse sections), the numbers of altered/degenerate fibres detected varied from one to approximately four. On a slide of peripheral nerve, one or two degenerate fibres were included in this grade.
Grade III	Disruption, fragmentation and distortion of a few axons, most of which were more intensely argyrophilic than the residual normal axons. Changes in myelin sheaths were minimal and usually consisted of small spheroids. In general, slides of cord with five or more, and slides of peripheral nerve with three or more degenerate fibres were recorded in this grade.
Grade IV	Qualitatively similar changes described for Grade V (see below) but affecting only moderate numbers of axons. Extent of change greater than in Grade III.
Grade V	Disruption, fragmentation and distortion of many axons, some of which are more argyrophilic on silver staining than normal axons. Considerable variation in thickness of affected axons with occasional large axon balls. Distortion and fragmentation of myelin sheaths in affected areas with variable numbers of myelinophages. A mild glial/Schwann cell response was occasionally present in the most severely affected areas. In general the extent/distribution was widespead.

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These samples were then processed through differing grades of Industrial Methylated Spirit toulene to paraffin.

Histological sections were reported as being cut at 8 um and stained with haematoxylin and eosin. Additional sections from each block were stained with Glees and Marsland method for axons* and with Solochrome Cyanin for myelin**.

* Marsland, T. A., Glees, P. and Erikson, L. B. 1954, J. Neuropath. Exp. Neurol. 14, 587.
** Page, K. M., 1970, J. Med. Lab. Technol. 27, 1

Slides were prepared from hens which survived the 90-day dosing period only. The hens which died were not included because of problems related to autolysis. The birds from groups 5 and 7 were not prepared for histology. The grading criteria used to assess neuropathology is appended. Grades I and II are considered to represent background variation.

The birds dosed with TOCP produced many instances of grade III, IV neuropathology in the sections prepared from the spinal cord to clearly indicate a neurotoxic effect of this agent.

There was a single incidence of grade III neuropathological gradings in the birds treated with pirimiphos-methyl (at 10 mg/kg/day). This was considered to be incidental.

5. Recovery - The birds in groups 9 and 10 which were set aside to recover from any effects of pirimiphos-methyl showed a regain in lost body weight. In some cases it took 4-16 days for the birds to recover from the signs of toxicity due to pirimiphos-methyl. No histopathology was conducted on the hens which were allowed to recover.

CONCLUSION: This study is CORE GUIDELINES. This study demonstrates that pirimiphos-methyl at dose levels up to and including 10 mg/kg/day for 90 doses does not induce a typical (TOCP type) delayed neurotoxicity. Although some signs of ataxia-like symptoms were noted, these were not followed up by histopathological findings. The TOCP treated hens had both well defined ataxia and clearly defined neuropathological lesions in the spinal cord sections.